Atty. Docket No.: 8039/1070 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Tomlinson, et al.	Examiner:	Ponnaluri, P.
Serial No.:	09/511,939		
Filed:	February 24, 2000	Group Art Unit:	1639
Entitled:	Method to Screen Phage Display Libraries with Different Ligands		
		Conf. No.:	5170

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF IAN M. TOMLINSON UNDER 37 C.F.R. §1.131

I declare:

1. I, Ian M. Tomlinson, am an inventor of the invention claimed in the above-noted U.S. Patent application.

2. I have read and understood the Office Action mailed July 11, 2003 and have read and understood the cited reference, U.S. Patent No. 6,057,098 (the "'098 patent;" issued to Buechler et al. on May 2, 2000 from an application filed April 4, 1997). I understand that the Examiner has cited the '098 patent as a novelty reference over claims 33-41 and 44-52.

The '098 patent is cited as teaching methods of producing a multivalent polypeptide display library comprising a library of phage representing tagged fusion proteins. The Examiner states that the tag can be any polypeptide with a known receptor showing high binding specificity for the tag (referring to column 7, lines 10-11). The Examiner further asserts that Buechler et al. teaches contacting the library with a receptor (which the Examiner characterizes as analogous to a generic ligand) and separating bound members of the library from unbound members to produce a sublibrary of polypeptides. The Examiner states that the selected sublibrary is then screened by contacting the library with a target and separating the library members bound to the target via their displayed polypeptides.

3. Prior to the April 4, 1997 filing date of the '098 patent, I had conceived of the invention as claimed in claims 33-41 and 44-52. The invention was reduced to practice with diligence shortly thereafter. The attached Exhibit 1 consists of copies of my notebook entries detailing the experiments that gave rise to the claimed invention. The dates on this exhibit have been redacted.

4. As documented in paragraphs A-N below, and in the accompanying exhibits, the object of the invention is to improve polypeptide library technology to increase the likelihood of identifying polypeptide molecules that bind desired targets. Many polypeptide libraries are made by introducing diversity into the binding site of a known polypeptide structure and typically this is performed using degenerate oligonucleotides and sometimes also the polymerase chain reaction. One of the problems facing those working with such libraries is that a large proportion of the polypeptides in the library are in fact non-functional and are unable to bind to a target ligand. Non-functional molecules can arise due to the introduction of amino acids into the binding site (encoded by the degenerate oligonucleotides) or in the framework regions (by errors caused by the PCR amplification process or in the oligonucleotides used to amplify the polypeptide-encoding genes) that prevent or perturb the ability of that molecule to fold properly. Alternatively, non-functional polypeptide molecules can be created by the introduction of frame-shifts or stop codons that prevent or perturb the expression of the polypeptide. The ratio of non-functional to functional members in a given library can result in a high background of non-functional polypeptide molecules during the isolation of functional polypeptides against a given target ligand Thus, a method that increases the proportion of functional and properly folded molecules in a library will increase the likelihood of identifying polypeptide molecules that bind a given target ligand. One area of polypeptide library technology that would benefit from a method that increases the proportion of functional and properly folded polypeptides is antibody library technology, because antibodies typically involve both heavy and light chain domains that each must be diversified and that each must properly fold in order to generate a functional binding molecule.

Prior to April 4, 1997, I conceived an approach to overcome this problem. In this approach, a sub-library of antibody Heavy chains is selected for members that properly fold, a sub library of Light chains is selected for members that properly fold, and the two selected sub-

libraries are combined to form a library of antibodies that have been pre-selected for folded members that is then selected for binding to target antigen. The pre-selection for proper folding is performed by binding the members of each sub-library to a generic ligand that only binds properly folded sub-library molecules. For example, the Heavy chain sub-library can be pre-selected with Protein A, and the Light chain sub-library can be pre-selected with Protein L. In one approach, scFV constructs are employed.

In order to select sub-libraries that properly fold, two scFv vector constructs are required:

a) a construct encoding a known "dummy" Heavy chain can be used to generate the sub-library of diverse Light chains; and

b) a construct encoding a known "dummy" Light chain can be used to generate the sub-library of diverse Heavy chains.

Following selection for proper folding of the diverse domains by generic ligand binding of both sub-libraries, the "dummy" domain of one sub-library is replaced with the corresponding folding-selected domains from the other sub-library to generate a library of diverse, properly folded scFvs that can be selected for target binding. Beginning before April 4, 1997, this approach was undertaken; reduction to practice by successful identification of scFv antibodies that bind test antigens was achieved thereafter following continued diligent efforts, described below and documented in the enclosed notebook page copies.

A) The first notebook entry (A), which occurred before April 4, 1997, documents the construction of the vectors for this approach. Specifically, the notebook entries state:

> "Construction of new vectors pCLEANV_H + pCLEAN$V\kappa$ for phage expression. These have dummy $V\kappa$ and V_H respectively in IT linker in vector pH (no Tags, no TAG, no pHEN seq site)."

Notebook entry A documents the conception of the claimed invention and a beginning of the reduction to practice by preparing the necessary dummy Heavy and Light chain vectors before April 4, 1997. The reference to "no Tags, no TAG" means that the encoded vectors do not encode epitope or other heterologous polypeptide tags, and they do not encode TAG stop codons. Vectors DP-47 and DPK-9 supplied the V_H and V_κ heavy and light chain antibody

frameworks, respectively. These V_H and V_κ frameworks are also useful in non-scFv antibody formats.

B) *Notebook entry (B),* shows the results of sequencing reactions, performed before April 4, 1997, to check the Heavy and Light chain dummy vectors. The entry documents the identification of VH2-4 as pCLEANVκ and Vκ1-5 as pCLEANV$_H$. Primary PCR amplifications for the library according to the invention were carried out on this date.

C) *Notebook entry (C)* documents the gel purification of the primary amplifications and the use of the isolated products for secondary amplifications to introduce diversity. The germline V gene segments DPK-9 (V_κ) and DP-47 (V_H) were used as templates for PCR mutagenesis. The different sub-libraries were made to include variability that mimics the introduction of diversity *in vivo* – the "primary" sub-libraries have diversity introduced in the H2, H3 and L3 loops, mimicking germline or junctional diversity; the "somatic" sub-libraries have diversity introduced in H1, H3, L1 and the end of L3 loops, mimicking diversity introduced as a result of somatic hypermutation. Diversity was introduced through use of amplification primers with the diversity codons "NNK" or "DVT." The "NNK" diversity codon encodes all 20 amino acids, including the TAG stop codon, but not the TGA or TAA stop codon. The "DVT" diversity codon encodes 22% serine and 11% tyrosine, asparagine, glycine, alanine, aspartate, threonine and cysteine, and closely mimics the distribution of amino acid residues in the antigen binding sites of natural human antibodies.

The differing mutagenic codon approaches produced four separate fragments for each of the NNK and DVT libraries: a "primary" V_H fragment (5A); a "primary" V_κ fragment (6A), a "somatic" V_H fragment (5B); and a "somatic" V_κ fragment (6B). Each of the amplified fragments was digested overnight with restriction enzymes for cloning.

Over the following days, the products of the overnight digestion were gel purified, and test ligations were set up using digested secondary amplification products and pCLEAN vector (See Notebook Entry D). Ligated DNAs were transformed into HB2151 E. coli cells. The results of the test ligations were used to set up main ligations for VH and VK libraries according

to the invention. The main ligations were transformed into HB2151 E. coli cells. Clones from the main ligations were assessed for insert.

D. The VH and VK libraries were then rescued to produce phage particles. *Notebook entry (D)* documents the generation of phage that display the molecules of the V_H and V_κ libraries on their surfaces. The ligations from Entry C were electroporated into *E. coli* strain HB2151. The resulting phage titers for sub-libraries (5A, 5B, 6A, 6B) are shown at the bottom of the page of Notebook Entry D.

E. The resulting phage libraries were then selected against Generic Ligands. *Notebook entry (E)* shows selection of the V_H sub-libraries 5A and 5B for folding by binding to Protein A, and the selection of the Vκ sub-libraries 6A and 6B by binding to Protein L.

Phage from each of the libraries were separately selected using immunotubes coated with 10 μg/ml of generic ligands Protein A and Protein L for the V_H and Vκ libraries, respectively. High titres for all four selected libraries indicate successful selection for functional/folded members. This demonstrates selection for function/folding against Generic Ligands.

F. DNAs from the libraries selected for function/folding and those not selected for function/folding were then prepared and cut with appropriate restriction enzymes. Vector and VH library selected for function/folding was test-ligated with insert comprising the VK library selected for function/folding. Also, Vector and VH library not selected for function/folding was test ligated with insert comprising VK library not selected for function/folding.

Also at this time, an ELISA screen was established for selection for function/folding. The screen involves a sandwich ELISA of Protein A on a plate. To establish the screen, the plate is overlaid with supernatant of dummy VH-VK library member (6A, 6B) or VH library member-dummy VK (5A, 5B) or VH library member-VK library member (NNK A, NNK B). Detection with Protein L-Horse radish peroxidase (HRP) indicated functional/folded VK library member, VH library member, or paired VH-VK library members, respectively.

Also during this time, main library ligations were performed, combining vector and VH library selected for function/folding with insert comprising VK library selected for

function/folding (Libraries C and D). This shows selection against a Generic Ligand to create a subset, followed by combination with selected subset from the other chain. Main library ligations combining vector and VH library that had not been selected for function/folding were test ligated with insert comprising VK library not selected for function/folding (Libraries E and F). *(See Notebook entry F)*. Resulting libraries were transformed into *E. coli*.

G. At this time, Library C had to be re-made due to a transformation failure (See *Notebook entry G*, top of page, stating "Library C remake").

H. Subsequent to the re-creation of Library C, clones from the VH-VK main library ligations (selected VH and VK) were tested for insert. VH-VK clones not selected against Generic Ligands were also tested for insert. *Notebook entry H, 2 pages* shows the results when clones from these libraries were expressed and tested in the ELISA function/folding screen. A high percentage of functional/folded clones was noted where there was pre-selection with the Generic Ligands compared with a low percentage in the absence of pre-selection with Generic Ligands. Specifically, *Notebook Entry H* shows that pre-selected Library C gives 81 of 96 positive and pre-selected Library D gives 91 of 96 positive, while non-selected Library E gives 34 of 96 positive and non-selected Library F gives 39 of 96 positive. It is also noted that the degree of binding is generally higher in the pre-selected clones. These data demonstrate that pre-selection with Generic Ligands enriches for functional/folded library members.

I. Subsequently, functional/folded and non-functional/folded clones from the screens were sequenced. All non-functional/folded clones sequenced had defects such as frame shifts or inappropriate stop codons that prevent function/folding.

Subsequently, new starting VH and VK libraries were test ligated into pCLEAN vectors and transformed into HB2151 E. coli cells.

At the same time, more functional/folded and non-functional/folded clones from the previous screen were sequenced. *Notebook entry I* (see lanes 1-20 in sequencing run NEW 18) shows the results of sequencing runs in which all folding/function-negative clones sequenced had defects (e.g., frame shift, TGA, TAG codons) that prevent function/folding. These results indicate that non-folding is not simply the result of non-expression. This is a demonstration that

selection with Generic Ligands selects for more than simply expression, and rather, selects for properly folded protein.

J. Subsequently, the new VH and VK libraries were rescued to produce phage particles. Protein A and Protein L were coated onto immunotubes and new VH and VK libraries according to the invention were selected for function/folding in the ELISA sandwich assay format. High titres for all four selected libraries indicate successful selection for functional/folded members.

Vector and the new VH library selected for function/folding was then test ligated with insert comprising the new VK library selected for function/folding.

New main library ligations combining vector and VH library selected for function/folding with insert comprising VK library selected for function/folding were then performed to establish Libraries G and H. Libraries G and H were transformed into E. coli. *Notebook entry J* shows resulting titres of libraries C, D, G and H (lower, right).

K. Subsequently, clones from the new main library were tested for insert (see *Notebook entry K*) and then expressed and tested in the function/folding ELISA screen. A high percentage of clones (72 of 96 with library G and 71 of 96 with Library H) was functional/folded where there was pre-selection with Generic Ligands, compared to a low percentage in the absence of pre-selection with Generic Ligands (see previous non-selected).

L. Phage resulting from folding/function pre-selected libraries were concurrently selected against Target Ligands. The Target Ligands included FITC-BSA, recombinant human leptin, thyroglobulin, BSA and hen egg lysozyme (HEL) – see *Notebook entry J.* Titres from the first round of selections are shown in the "r1 selections" table on *Notebook Entry J.*

M. Titres from a second round of selections against Target Ligands ("r2 selections") are shown in *Notebook entry L.* Titres for a third round ("r3 selections") are shown in *Notebook Entry M.*

N. Target Ligand binding assays were then performed using clones from the third round of Target selection. *Notebook entry N* shows the results of target binding assays of monoclonal VH-VK pairings to the target ligands in assays using the immobilized target ligands and labeled

Generic Ligand for detection. Positive clones represent the successful selection of polypeptides that bind to Target and Generic Ligands. This represents a full reduction to practice of the claimed invention. This successful reduction to practice was the result of continuous diligent efforts from before the April 4, 1997 date of the Buechler et al. reference.

5. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

12 SEPT ' 2003

Date

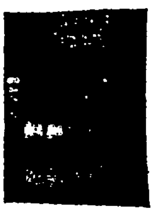
Ian M. Tomlinson

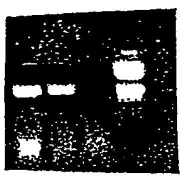
EXHIBIT 1

TEBOOK ENTRY A

Construction of new vectors ie pCLONVH + pCLONVK for phage expression - these have dummy VK and VH respectively in IT linker in vector pH (note Tags, no TAG, no pHEN seq site).

pH sequence checked using LMB3 - OK!
cut SfiI/NotI O/N + ligate 'INSERT'
24 colonies checked - all +ve
Take one of these cut NcoI/XhoI + SalI/NotI
PCR DP-47 + DPK9 with FR1B primers + CDR3 primers for dummy chains. (DP-47 good with Pfu, DPK9 not - used Taq)
PCR prep these + re PCR with 5F/B + 6F/B respectively (25 cycles)





cut amplified bands as above - ligate + transform into TG-1
Check ~~sequences~~ for insert (see next page)

VH screen LMB3 link seq
VK screen DPK9 FR1 seq, GIII fwd LMB2
BOTH LMB3 GIII fwd LMB2

(Mix up but VH2 + VK1 good - check 6x sequences)
VH2-4 IS good
sequences for VK1-3, 4, 5 not long enough
use other primers on miniprep template.

ille

NOTEBOOK ENTRY B

Recheck of pCLEAN VECTORS NOV 16

WAS		LMB3	rc linkseq	rc G3fwd	rc fdseq1	10 pκ9 fR1seq
(3)	VH2-4 NO ERRORS ✓✓✓	1	6	11	16	
(9)	VK1-3 1 SILENT MUTATION TGT→TGC	2	7	12	17 DMDS	21 DMDS
(10)	VK1-4 ✓✓ NO ERRORS	3	8	13	18 "	22 "
(11)	VK1-5 ✓✓ NO ERRORS	4	9	14	14	23 "
	pIT3	5	10	15	20	

VH2-4 is pCLEAN VK

~~[illegible]~~

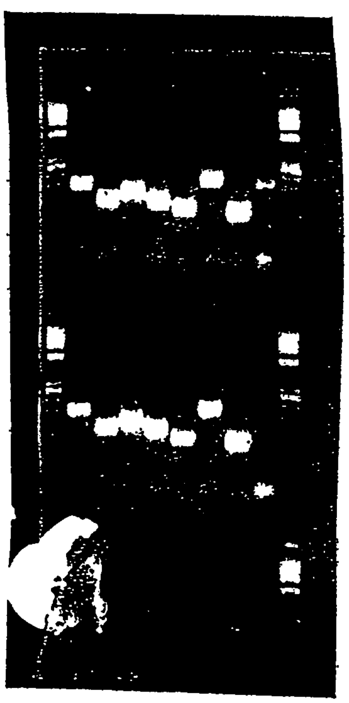
VK1-5 is pCLEAN VH.

pIT3 is GOOD!

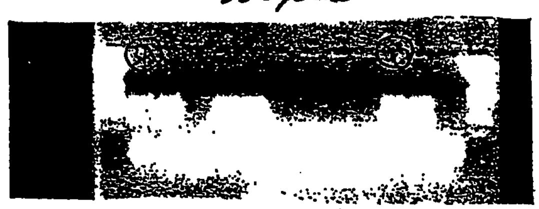
SYGAN library construction

Various 1° PCRs performed (conditions as NNK lib)

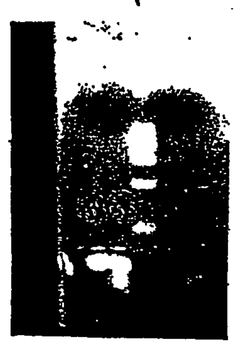
30 cycles



25 cycles

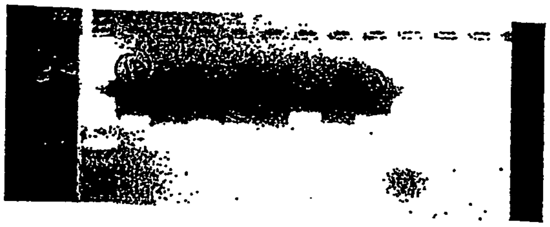


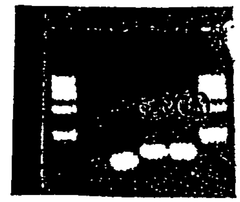
20 cycles



These gel purified + cleaned up. (1A, 2A, 3A, 4A → electroelution then EtOH ppt
5A, 7A · P/C ×2 EtOH ppt
6A, 8A Gel extraction)

NOTEBOOK ENTRY C





These mixed and amplified according to NNK protocol
(5µl used per PCR) (20 cycles with PR
1st 5 cycles without)

pCLEAN VH/VK miniprep

VH	5A	used 1A + 2A	(1+2)
	5B	used 1B + 2B	(5+6)
VK	6A	used 3A + 4A	(3+4)
	6B	used 3B + 4B	(7+8)

Test Ligations

NOTEBOOK ENTRY D

	V	INS	H_2O	cols	INS (PCR screen)	
1	1	5	0	130	23/24	20µl total
2	1	2	3	140	23/24	2 BUFF
3	1	0.5	4.5	156	20/24	0.5 LIGASE
4	1	0.1	4.9	99	15/24	11.5 H_2O
5	1	0	5	0		
	-ve control			0		

SYGON LIB PREP ↓

Master ligations use 1V : 21 100µl.

LIB MADE IN HB2151.

		cols on LIB SIZE 10^4	partial TOTAL		% INS	% ACT SIZE	
5A SYG	5A	28	9.6×10^5 (×10.6)	1.02×10^7	23/24	9.78×10^6	10µl V
5B SYG	5B	18	7.2×10^5	7.63×10^6	23/24	7.31×10^6	20µl I
6A SYG	6A	21	8.4×10^5	8.90×10^6	24/24	8.90×10^6	10µl BUFF
6B SYG	6B	16	6.4×10^5	6.78×10^6	24/24	6.78×10^6	3µl LIGASE
							57µl H_2O

~~[illegible]~~ nH = 1.2×N 10 [illegible] ... [illegible]

DNA Prepped from these   1ml glycerol → 40ml 2XTY
Prepped with Qiagen mini prep

Also inoculated 100µl into 100ml 2XTY AMP GLU
PA/PL coated O/N (10µg/ml in PBS) for selection.

(14.75 ml total)   STANDARD WASH CONDITIONS 20x 20x.

	COLS ON 10^5	TITRE (IN 14.75mls) ×2.95×10^6
5A	460	1.36×10^9
5B	541	1.60×10^9
6A	518	1.53×10^9
6B	304	8.97×10^8

cells were -ve.

DNA Prepped with Qiagen midiprep kit - resuspend in .
125µl TOTAL
10mM TRIS

40µl of this cut in 80µl reaction
8 Sal I BUFF
2 Sal E
2 Not I
28 H_2O
40 DNA

Also uncleaned cut
Trial ligations SYGA, SYG AC

20µl

1 LIGASE
2 BUFF
+ 14 H_2O

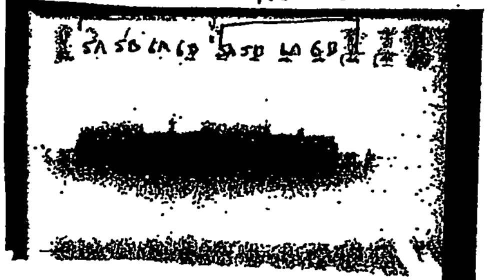
		SAV	6A1	H_2O	COLS
SYG AC	1	0.5	0	~~14.5~~ 2.5	+
	2	0.5	0.1	~~14~~ 2.6	30×5
	3	0.5	0.5	~~13~~ 2	
SYG A	4	0.5	2.5	~~14~~ 0	← BEST
	5	1	0	14.5 2.5	++ 100×5
	6	1	0.1	~~14~~ 2.4	
	7	1	0.5	~~13~~ 2	

MORE (lots) COLS ON SYG A THAN SYG AC!
Use $\boxed{1:3}$

BIG LIB ligations 100 µl

10 BUFF
10 Vector (5A cuts)
20 INSERT (6A cut)
4 LIGASE
~~56~~ H2O.

CLEAN NO CLEAN



After gel extraction.

8x BLASTS each library (0.5 µl) Add 1.5 µl 2xTY
Pool cell (1.6 mls) → (160 µl → 2 ml) → (200 µl → 2 ml) → (200 µl → 2 ml)

		LIB	10	10²	10³		
[illegible]	LIB	COLS ON 10⁴	TOTAL SIZE (LIB, 10, 10², 10³)	10² SIZE (10, 10², 10³)	10² SIZE (10², 10³)	10³ SIZE (10³)	INSERT %
C	A CLEAN	255	8.16 × 10⁷	8.16 × 10⁶	8.16 × 10⁵	8.16 × 10⁵	24/24 100
D	B CLEAN	744	2.38 × 10⁸	2.38 × 10⁷	2.38 × 10⁶	2.38 × 10⁵	24/24 100
E	A	781	2.5 × 10⁸	2.5 × 10⁷	2.5 × 10⁶	2.5 × 10⁵	23/24 95.8
F	B	706	2.26 × 10⁸	2.26 × 10⁷	2.26 × 10⁶	2.26 × 10⁵	23/24 95.8

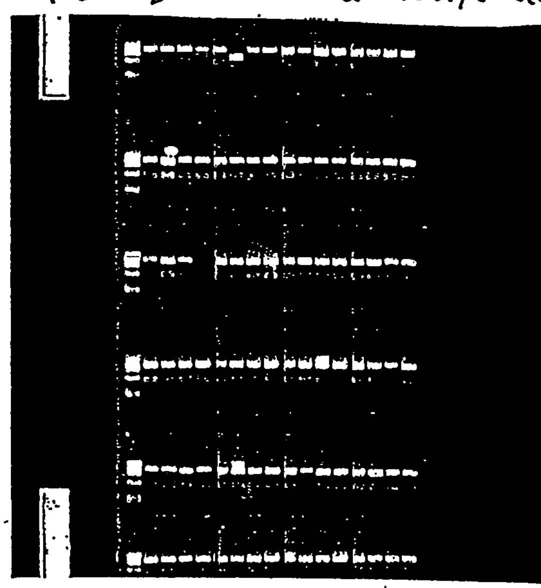
CONFIRM! Use 1/10 of 10²

AGAROSE DROPPED OFF PLATE

– is – ve

pH has 20 on 10² 1.2 × 10⁸

– TITRES CAN INCREASE
redo 20x BLASTS with new/old ligations.



LIB C remake (A cleaned S7G (DVJ))

COLS ON 10^4 DNA SIZE (NEAT, 10, 10^2, 10^3) 10 SIZE (10, 10^2, 10^3) 10^2 SIZE (10^2, 10^3) 10^3 SIZE (10^3)

1024 5.57×10^8 5.57×10^7 $5.5\cancel{8} \times 10^6$ 5.57×10^5 4H24!

THIS IS C-2. -ve's-ve!

C-2, D, E, F DNA Prepped + cut Nco/Not (NEB 3 + BSA)

LMP Gel → Gel extract → ligate into pAB1.

TEST selections with C2, D2, E2, F2, C3, D3, E3, F3

Make phage, select on Immunotubes - (25 μg/ml NIP BSA)

MOLECULAR DEVICES
Raw Data (Plate)

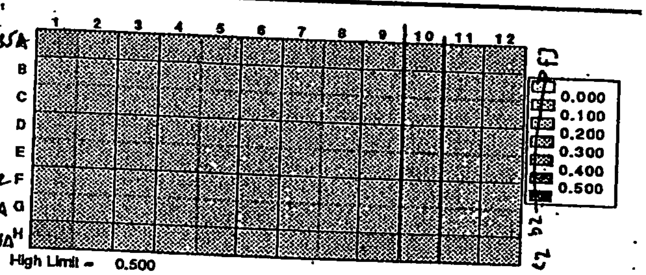
DATA FILE: c/d/e/f unsel
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 20.80°C
CALIBRATION: ON
SET TEMP: OFF

Optical Density

ANT (μg/ml) CONC		1 C2	2 D2	3 E2	4 F2	5	6 C3	7 D3	8 E3	9 F3	10	11 NO ANTIGEN	12	
2.5	A	0.214	0.124	0.181	0.126	0.045	0.082	0.111	0.181	0.035	0.140	0.049	0.166	NIP BSA
5	B	0.032	0.027	0.018	0.024	0.019	0.016	0.018	0.032	0.044	0.039	0.047	0.019	BIP
10	C	0.073	0.104	0.042	0.046	0.040	0.041	0.021	0.014	0.019	0.023	0.091	0.076	UBI
20	D	0.149	0.122	0.050	0.084	0.074	0.077	0.111	0.014	0.087	0.050	0.029	0.020	THY
00	E	0.077	0.037	0.013	0.009	0.010	0.040	0.011	0.028	0.024	0.023	0.044	0.098	BSA
10	F	0.014	0.021	0.014	0.028	0.013	0.013	0.012	0.019	0.017	0.018	0.015	0.019	PERRF
20	G	0.054	0.010	0.020	0.030	0.011	0.009	0.041	0.084	0.018	0.026	0.054	0.052	OX BSA
20	H	0.229	0.175	0.037	0.013	0.112	0.082	0.084	0.130	0.221	0.120	0.137	0.119	FITC BSA

MOLECULAR DEVICES
Gray Scale (Plate)

DATA FILE: c/d/e/f unsel
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 20.80°C
CALIBRATION: ON
SET TEMP: OFF



High Limit = 0.500
Low Limit = 0.000

10μl unselected phage against
No real evidence for specific binding

cols on 10^2 = 30,000

For test selections

	cols on 10^2			cols on 10^2	
C2	247	7.41×10^6	C3	92	2.76×10^6
D2	212	6.36×10^6	D3	243	7.29×10^6
E2	165	4.95×10^6	E3	158	4.74×10^6
F2	53	1.59×10^6	F3	154	4.62×10^6

-ve's-ve

Make phage do ELISA

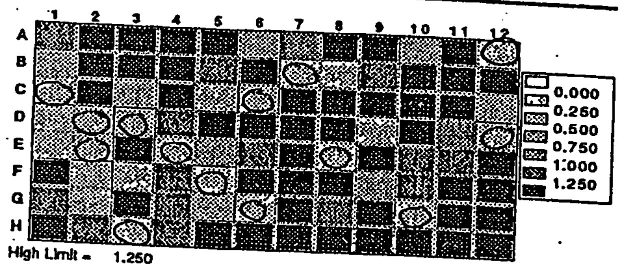
DEF-ves ○ SCONUOF < .1 ON ~~...~~ ~~...~~ (dephes) H 1151 50ul
7.1 ON BOTH the ... ... 2ug/ml PL d. plate Supe
1:5000 PA-HRP

DATA FILE: C 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 21.70°C
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.191	1.280	1.524	1.413	1.433	0.413	0.823	1.600	1.472	0.341	1.372	0.045
B	0.494	1.392	1.376	1.422	1.228	1.435	.039	0.218	1.001	1.308	1.268	1.294
C	0.02	1.278	0.278	1.437	0.146	0.052	1.480	1.343	1.442	1.346	1.320	0.368
D	0.183	0.031	.027	1.124	1.250	1.476	1.270	1.283	0.110	1.285	0.999	.044
E	0.083	0.032	1.461	.049	0.648	1.149	1.319	0.027	1.291	1.244	1.143	1.450
F	1.380	0.207	0.198	1.042	0.032	1.545	1.333	1.401	0.544	1.200	1.384	1.474
G	1.139	0.239	1.339	1.220	0.432	.04	1.481	1.012	1.268	0.044	1.375	1.326
H	1.293	1.106	0.030	1.177	1.308	1.415	1.414	1.273	1.435	1.384	1.269	1.330

DATA FILE: C 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 21.70°C
CALIBRATION: ON
SET TEMP: OFF



High Limit = 1.250
Low Limit = 0.000

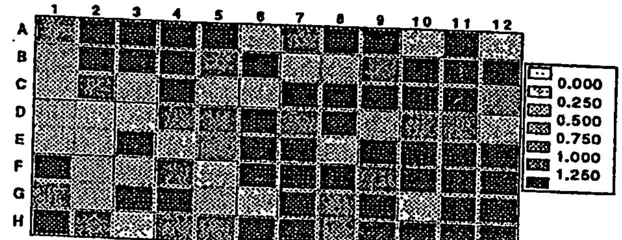
89/96 +ve.

DATA FILE: C 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 21.90°C
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.230	1.280	1.365	1.361	1.378	0.129	1.064	1.496	1.521	0.226	1.465	0.03
B	0.588	1.291	1.284	1.305	1.147	1.326	0.032	0.387	1.172	1.361	1.359	1.423
C	0.031	1.240	0.340	1.391	0.217	0.055	1.418	1.363	1.491	1.321	1.363	0.800
D	0.265	0.049	.032	1.007	1.203	1.431	1.078	1.296	0.400	1.245	1.026	0.054
E	0.108	0.037	1.405	0.084	0.965	1.289	1.319	0.031	1.422	1.403	1.340	1.580
F	1.401	0.381	0.258	1.066	.041	1.611	1.308	1.469	1.115	1.420	1.620	1.475
G	1.157	0.381	1.286	1.299	0.455	.03	1.495	1.127	1.367	0.073	1.448	1.401
H	1.261	1.132	0.044	1.212	1.236	1.305	1.259	1.117	1.306	1.288	1.279	1.359

DATA FILE: C 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 21.90°C
CALIBRATION: ON
SET TEMP: OFF



High Limit = 1.250
Low Limit = 0.000

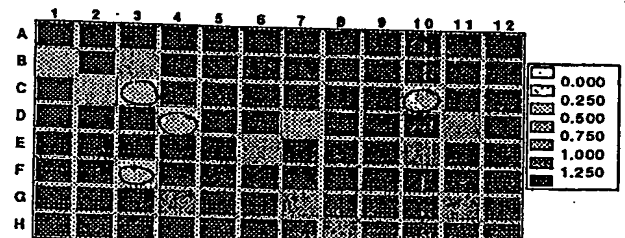
DATA FILE: D 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 22.00°C
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.623	1.634	1.578	1.447	1.356	1.492	1.437	1.320	1.291	1.458	1.412	1.408
B	0.263	1.494	0.910	1.342	1.626	1.379	1.328	1.416	1.462	1.410	1.411	1.362
C	1.394	0.663	0.027	1.455	1.539	1.305	1.429	1.476	1.301	0.027	1.466	1.485
D	1.610	1.522	1.541	.027	1.418	1.447	0.382	1.473	1.506	1.400	0.920	1.365
E	1.574	1.480	1.438	1.318	1.489	0.840	1.442	1.363	1.312	1.001	1.352	1.326
F	1.613	1.459	0.029	1.412	1.307	1.509	1.264	1.407	1.428	1.449	1.308	1.271
G	1.378	1.430	1.486	1.170	1.382	1.501	1.235	1.437	1.438	1.474	1.224	1.402
H	1.563	1.355	1.584	1.474	1.446	1.331	1.516	1.115	1.358	1.338	1.369	1.368

91/96 +ve

DATA FILE: D 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 22.00°C
CALIBRATION: ON
SET TEMP: OFF



High Limit = 1.250
Low Limit = 0.000

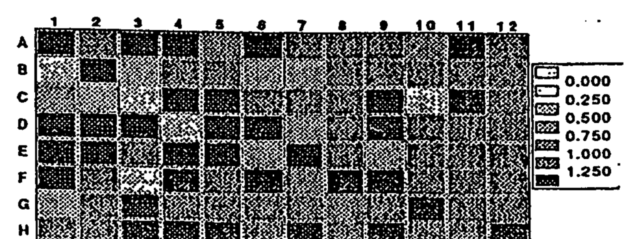
DATA FILE: D 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 22.00°C
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.264	1.224	1.303	1.305	0.928	1.283	1.245	1.161	1.078	0.987	1.283	1.166
B	0.093	1.348	0.692	1.123	1.217	0.620	0.716	1.146	1.233	1.071	1.248	1.146
C	0.852	0.637	.031	1.426	1.328	1.206	1.214	1.213	1.289	0.04	1.323	1.221
D	1.404	1.424	1.336	.03	1.297	1.298	0.587	1.210	1.427	1.091	1.160	1.146
E	1.282	1.310	1.211	1.375	1.318	0.748	1.288	1.042	0.582	1.142	1.164	1.199
F	1.382	1.093	.029	1.353	1.184	1.338	0.961	1.261	1.289	1.236	1.228	1.036
G	0.618	1.177	1.257	1.090	1.009	1.237	1.123	0.824	1.132	1.277	1.166	1.139
H	1.219	1.205	1.509	1.355	1.328	1.187	1.366	1.079	1.376	1.230	1.028	1.295

V.

DATA FILE: D 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 22.00°C
CALIBRATION: ON
SET TEMP: OFF



High Limit = 1.250

DATA FILE: E 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.10°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.037	0.021	0.038	0.020	0.036	0.389	0.078	0.108	0.091	0.133	0.407	0.067
B	1.252	0.205	0.025	0.025	0.02	1.556	0.180	0.020	0.747	1.051	0.031	1.439
C	1.678	0.582	0.820	0.02	0.06	0.025	0.03	0.080	0.02	1.499	1.393	0.036
D	0.025	0.027	0.040	0.064	0.029	0.026	0.022	0.528	0.100	0.048	0.948	0.039
E	0.037	0.080	0.168	0.079	0.033	1.204	0.057	0.032	0.091	0.029	0.055	1.186
F	0.872	1.306	0.087	0.025	0.02	0.080	0.150	0.067	0.030	0.038	0.840	0.037
G	0.073	0.738	0.12	1.418	0.803	1.448	0.862	0.299	1.314	0.11	0.027	0.484
H	0.802	0.057	0.041	1.477	0.041	1.123	0.019	1.265	0.020	0.067	0.020	0.130

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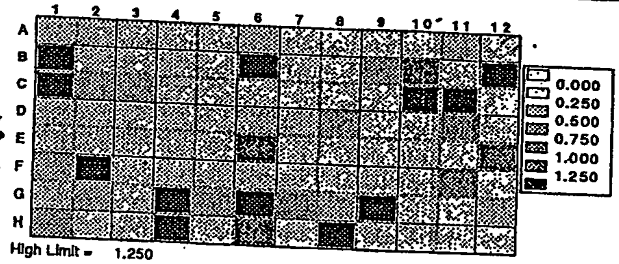
DATA FILE: E 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.10°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF



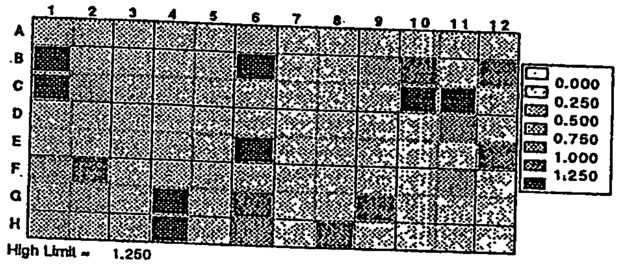
High Limit = 1.250
Low Limit = 0.000

DATA FILE: E 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.10°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.035	0.026	0.102	0.026	0.036	0.258	0.034	0.047	0.081	0.108	0.087	0.043
B	1.259	0.034	0.035	0.028	0.025	1.351	0.121	0.025	0.714	1.064	0.029	1.238
C	1.582	0.874	0.123	0.037	0.046	0.026	0.035	0.036	0.026	1.338	1.372	0.033
D	0.029	0.029	0.041	0.046	0.024	0.038	0.026	0.136	0.074	0.049	0.769	0.049
E	0.047	0.056	0.175	0.049	0.032	1.272	0.070	0.035	0.062	0.031	0.065	1.223
F	0.871	1.229	0.032	0.029	0.027	0.035	0.225	0.113	0.025	0.025	0.642	0.030
G	0.038	0.848	0.030	1.358	0.173	1.241	0.033	0.108	1.004	0.054	0.024	0.034
H	0.307	0.056	0.037	1.375	0.047	0.953	0.025	1.038	0.027	0.033	0.027	0.039

DATA FILE: E 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.10°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF



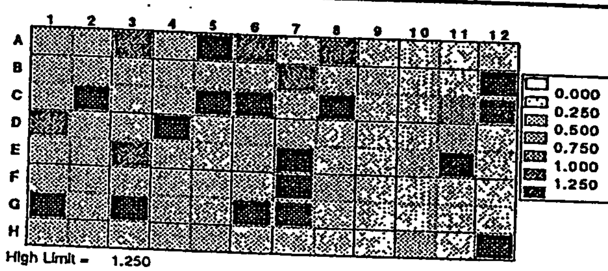
High Limit = 1.250
Low Limit = 0.000

DATA FILE: F 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.10°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.380	0.042	1.146	0.195	1.430	1.018	0.081	1.099	0.037	0.131	0.086	0.247
B	0.381	0.047	0.043	0.030	0.065	0.213	1.125	0.129	0.031	0.032	0.238	1.449
C	0.039	1.481	0.028	0.202	1.417	1.459	0.141	1.488	0.024	0.03	0.850	1.871
D	1.226	0.044	0.029	1.509	0.040	0.260	0.028	0.182	0.228	0.026	0.330	0.066
E	0.039	0.071	1.183	0.03	0.034	0.045	1.349	0.025	0.123	0.398	1.327	0.208
F	0.084	0.037	0.053	0.033	0.036	0.094	1.377	0.383	0.029	0.027	0.044	0.021
G	1.823	0.047	1.269	0.035	0.042	1.272	1.305	0.024	0.036	0.020	0.033	0.02
H	0.03	0.073	0.03	0.037	0.362	0.029	0.112	0.021	0.088	0.378	0.138	1.493

DATA FILE: F 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.10°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF



High Limit = 1.250
Low Limit = 0.000

DATA FILE: F 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.10°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.217	0.069	0.493	0.044	1.393	0.678	0.033	0.741	0.03	0.129	0.083	0.181
B	0.083	0.038	0.03	0.024	0.057	0.125	0.798	0.067	0.04	0.03	0.122	1.210
C	0.04	1.28	0.02	0.087	1.317	1.309	0.089	1.341	0.030	0.04	0.846	1.491
D	0.797	0.03	0.035	1.245	0.037	0.165	0.036	0.239	0.173	0.029	0.192	0.07
E	0.04	0.047	1.039	0.032	0.044	0.04	1.287	0.03	0.09	0.198	1.146	0.193
F	0.03	0.047	0.034	0.03	0.038	0.083	1.240	0.284	0.038	0.031	0.034	0.027
G	1.176	0.040	0.886	0.03	0.03	1.012	1.070	0.026	0.034	0.028	0.029	0.02
H	0.02	0.043	0.032	0.032	0.108	0.037	0.05	0.029	0.04	0.118	0.053	1.259

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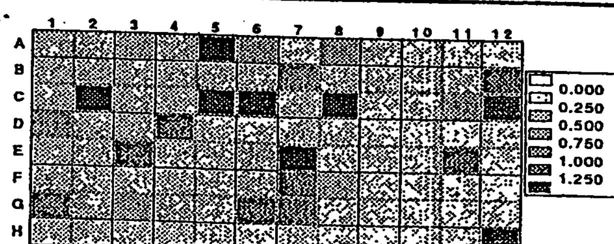
DATA FILE: F 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.10°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF



High Limit = 1.250
Low Limit = 0.000

NOTEBOOK ENTRY H CONTINUED

SEQUENCING (NEW #9.

	VH	L	VK	L	(NSW 18) Reren	DN correct VH	VK
NNKA + E1	linksequal	LMB2		17	This must come under LMB 21	✓	✓
NNKA - E2	"	2	"	18	22 FS IN VK CDR3	✓	✓
NNKB + A2	"	3	"	19	23 FS IN VH FR 4 → STILL GIVES for ELISA	✓	✓
NNKB - A1	"	4	"	20	TAG 24 IN VH CDR1 ! FS IN VK FR4	✓	✓
C + A1	"	5	"	21	25	✓	✓
C - B7	"	6	"	22	FS 26 IN VH CDR 2	✓	✓
b + A1	"	7	"	23	27	✓	✓
b - C3	"	8	"	24	28 FS IN VK CDR1	✓	✓
E + B1	"	9	"	25	29	✓	✓
E - A2	"	10	"	26	FS 30 IN VH CDR 2.	✓	✓
F + A3	"	11	"	27	31	✓	✓
F - A2	"	12	"	28	GLLIB 32 !! FS IN VK FR1 ~~R...~~	X	✓
D3 NIP-BSA r2 - D8	"	13	"	29	33 K IN ALL POSN 35 of VH!	- ✓	- ✓
r3 - D8	"	14	"	30	34	✓	✓
r3 - D11	"	15	"	31	35	✓	✓
r3 - D12	"	16	"	32	36	- ✓	- ✓

PCOG1 B1P3 B1P A1 — pHENseq 33 QQYRFGPRT

M1 — " 34 QQ RLRPLT

C2 NIP-BSA r3 - A5 — linkseq on 35 — RIPARGTUTH GGLR

C3 NIP-BSA r3 - B3. — " 36 — " GGLR

N.B TEMPLATE NNKB A2 is fine but has FS IN VH FR 4

THIS WILL BE ELIMINATED BY PHESCREEN!

Selections Part II

	STOCK CONC	FINAL CONC	USE IN 3ml (PBS)
FITC-BSA(FLUKA)	5.7mg/ml	20µg/ml	~~10.53~~µl
LEPTIN (rh)	3.3mg/ml	50µg/ml	45.45µl
THYROGLOBULIN	1mg/ml	50µg/ml	150µl
BSA	10mg/ml	100µg/ml	30µl
HEL	18.4mg/ml	500µg/ml	81.52µl

LIBS C, D, G + H Prepared as follows
400µl (G/H) or 200µl (C/D) of glycerol stock → 250ml 2xTY 100µg/ml AMP 1% Glu

Grown till OD = 0.5 Add 50µl VCS stock to 50ml culture
Incubate 30 minutes at 37°C (waterbath) Spin + resuspend in
250ml 2xTY, 100µg/ml AMP, 50µg/ml KAN, 0.2% Glu. Grow ON at 30°C.

200ml of this spun → supe to PEG ppt x2 → 10ml PBS
0.5ml C added to 0.5ml G select x5
0.5ml D added to 0.5ml H select x5

TITRES ON INPUT

	COLS ON 10^4 dil	PHAGE/ml
C	79	1.58×10^{13}
D	91	1.82×10^{13}
G	45	9.00×10^{12}
H	87	1.74×10^{13}

CG INPUT = 1.24×10^{13}/ml
DH INPUT = 1.78×10^{13}/ml
CG SIZE = 1.48×10^{9}
DH SIZE = 1.24×10^{9}
EQUIV SIZES comp to AE 1.46×10 [illegible]
BR 6.39×1[illegible]
reflected at clear.

selections

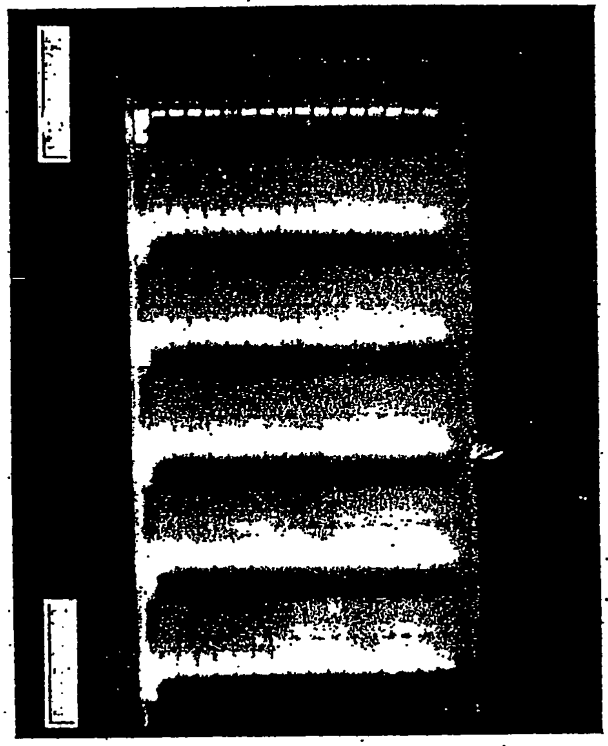
	cols on nodil	TITRE
10 CG	240	7.06×10^{6}
11 CG	132	3.88×10^{6}
12 CG	260	7.64×10^{6}
13 CG	300	8.82×10^{6}
14 CG	74	2.18×10^{6}
10 DH	234	6.88×10^{6}
11 DH	254	7.47×10^{6}
12 DH	146	4.29×10^{6}
13 DH	102	3.00×10^{6}
14 DH	68	2.00×10^{6}

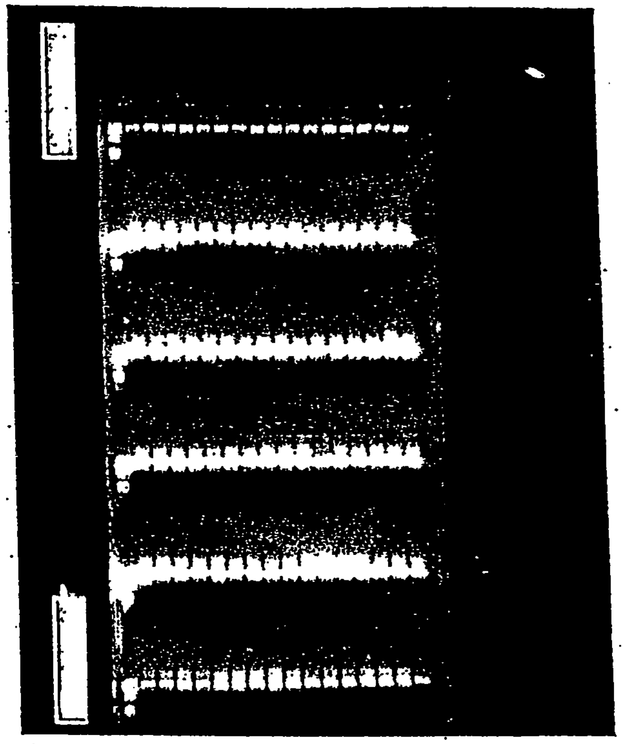
NOTEBOOK ENTRY K

Pk/PA assay on libs G+H (pABI HB215.1 clones)

PCR

LIB G   LIB H



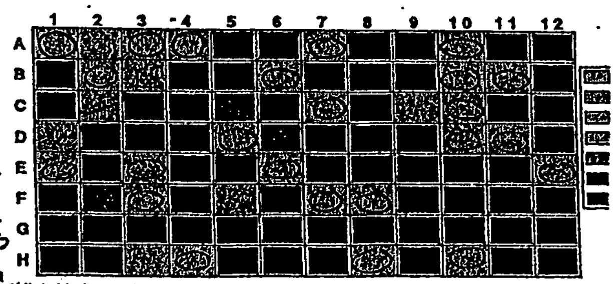


DATA FILE: G 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.   AUTOMIX: OFF
WAVELENGTH: 450-650   CALIBRATION: ON
MEAN TEMP: 22.30°C   SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.098	0.456	0.100	0.106	2.494	1.692	0.097	1.057	2.451	0.100	2.324	2.192
B	2.360	0.099	0.503	1.942	1.379	0.089	2.058	2.250	2.098	0.101	0.101	2.106
C	2.351	0.471	1.162	2.267	0.938	1.994	0.100	2.192	0.151	0.109	2.044	2.251
D	0.434	2.049	2.448	2.066	0.120	0.995	2.397	2.433	1.981	0.108	0.110	2.417
E	0.109	2.247	0.116	1.954	2.414	0.402	2.213	2.282	2.330	1.650	2.335	0.117
F	1.840	1.011	0.127	2.143	0.273	2.181	0.102	0.096	2.222	2.164	2.182	2.573
G	2.097	1.987	1.790	2.310	2.315	2.319	2.291	2.464	1.955	2.481	2.569	2.167
H	2.589	1.750	0.367	0.107	2.443	1.870	1.851	0.114	2.304	0.140	1.319	2.393

DATA FILE: G 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.   AUTOMIX: OFF
WAVELENGTH: 450-650   CALIBRATION:
MEAN TEMP: 22.30°C   SET TEMP: OFF



72/96 +ve

High Limit = 1.250
Low Limit = 0.100

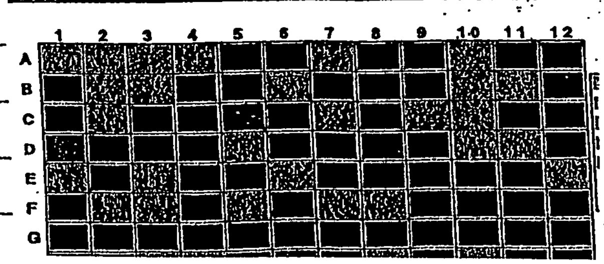
DATA FILE: G 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.   AUTOMIX: OFF
WAVELENGTH: 450-650   CALIBRATION: ON
MEAN TEMP: 22.50°C   SET TEMP: OFF

75%

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.098	0.273	0.088	0.129	2.589	1.607	0.087	1.118	2.375	0.093	2.110	2.184
B	2.112	0.105	0.475	2.022	1.557	0.098	1.984	2.120	2.345	0.100	0.088	2.308
C	2.244	0.499	1.177	2.246	0.869	1.987	0.098	2.107	0.163	0.103	2.195	2.141
D	0.899	2.153	2.271	1.940	0.103	1.147	2.294	2.363	1.909	0.095	0.105	2.309
E	0.151	2.088	0.136	1.899	2.254	0.108	2.167	2.185	2.323	1.793	2.207	0.113
F	1.767	0.782	0.159	2.019	0.277	2.100	0.111	0.112	2.121	2.170	2.023	2.437
G	[illegible]	[illegible]	1.826	2.253	2.298	2.336	2.154	2.299	2.016	2.473	2.042	2.237

DATA FILE: G 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.   AUTOMIX: OFF
WAVELENGTH: 450-650   CALIBRATIC
MEAN TEMP: 22.50°C   SET TEMP:

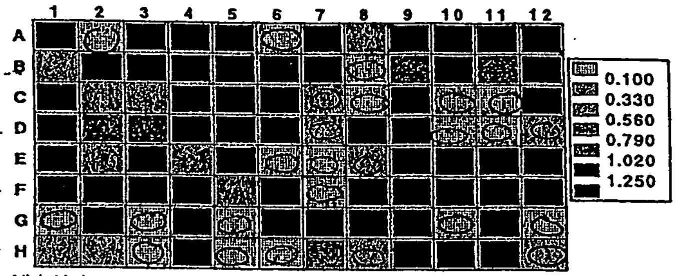


DATA FILE: H 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.80°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.958	0.079	1.456	2.049	1.906	0.088	2.042	0.251	2.261	2.027	2.089	2.265
B	0.317	1.248	1.756	1.947	2.070	2.157	1.976	0.086	0.995	1.924	0.968	2.019
C	1.627	0.319	0.269	1.913	1.448	1.901	0.101	0.085	2.037	0.090	0.089	1.691
D	2.166	1.002	0.976	1.964	2.019	2.047	0.137	1.604	2.121	0.077	0.080	0.100
E	2.530	0.164	2.146	0.155	2.010	0.091	0.081	0.136	2.264	1.855	1.118	2.007
F	1.441	2.118	2.038	2.110	0.655	1.670	0.097	2.134	2.140	1.650	1.478	1.963
G	0.084	1.936	0.084	1.341	0.092	1.892	2.400	2.178	2.084	0.083	2.393	0.089
H	0.194	0.421	0.083	1.906	0.091	0.093	0.928	0.121	1.708	2.098	1.614	0.130

DATA FILE: H 1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.80°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF



High Limit = 1.250
Low Limit = 0.100

DATA FILE: H 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.80°C.
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

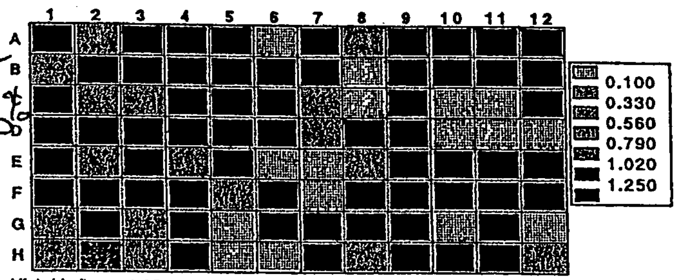
Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.003	0.103	1.598	2.140	1.920	0.094	2.056	0.459	2.194	1.991	1.988	2.389
B	0.362	1.588	1.984	2.083	2.111	2.220	1.965	0.098	1.768	2.159	1.140	2.020
C	1.734	0.461	0.556	1.993	1.701	2.041	0.116	0.087	2.023	0.085	0.082	1.962
D	2.106	1.576	1.623	2.225	2.165	2.115	0.186	1.775	2.177	0.074	0.079	0.087
E	2.545	0.347	2.120	0.160	2.129	0.089	0.091	0.118	2.293	2.096	1.244	2.106
F	1.674	2.301	2.065	2.247	0.763	2.099	0.086	2.058	2.219	1.935	1.637	2.090
G	0.105	2.048	0.100	1.644	0.096	2.168	2.418	2.137	2.216	0.074	2.363	0.087
H	0.344	0.858	0.107	2.175	0.092	0.090	1.248	0.131	1.930	2.140	1.569	0.151

71/96 +ve

73.6%

DATA FILE: H 2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.80°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF



High Limit = 1.250
Low Limit = 0.100

SCORE < 0.150 ON ~~BOTH~~ EITHER -ve

> 0.150 ON BOTH +ve

r2 SELECTIONS.

COLS. ON 10^2 (5 ⇒ 100 μls 50) SIZE

10CG	84	6.72×10^5
11CG	506	4.05×10^6
12CG	3	$\boxed{2.40 \times 10^4}$ seems v. low (?) redo? if r3 poly phage -ve
13CG	23	1.84×10^5
14CG	116	9.28×10^5
10DH	124	9.92×10^5
11DH	346	2.77×10^6
12DH	55	4.40×10^5
13DH	206	1.65×10^6
14DH	562	4.50×10^6

[redacted]

NOTEBOOK ENTRY M

r3

	CCLS on 10^2	SIZE	ANTIGEN
10 CG	4	3.2×10^4	FITC-BSA
11 CG	159	1.27×10^6	LEPTIN
12 CG	~800	6.40×10^6	THYROGLOBULIN
13 CG	45 (on 10^4)	3.60×10^7	BSA
14 CG	44	3.52×10^5	HEL
10 DH	9	7.20×10^4	FITC-BSA
11 DH	14	1.12×10^5	LEPTIN
12 DH	24	1.92×10^5	THYROGLOBULIN
13 DH	28 (on 10^4)	2.24×10^7	BSA
14 DH	1	8.00×10^3	HEL

All r3 phage (except 11CG had an awful lot of phage - possible contaminant)
BSA binders from CG + old libs → no evidence (yet!) (for: genes grown?)
binders from other selections

Make r3 phage + do 2 × each r3 monoclonals

NOTEBOOK ENTRY N

ISAS

FITC/leptin poly

Dual End. AUTOMIX: OFF
450-650
20.30°C
CALIBRATION: ON
SET TEMP: OFF

DATA FILE: FITC/leptin poly
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 20.30°C
CALIBRATION: ON
SET TEMP: OFF

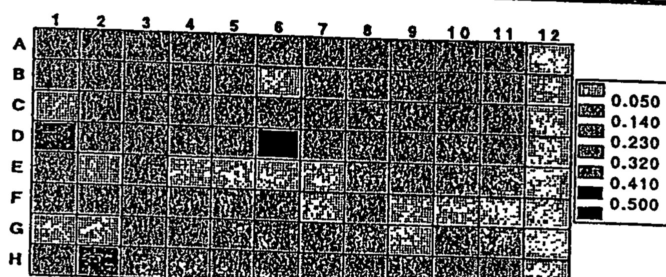
Optical Density

13CC 14CC 10DH 11DH 12DH 13DH 14DH /

3	4	5	6	7	8	9	10	11	12
40	0.210	0.069	0.053	0.211	0.164	0.099	0.288	0.217	0.006
17	0.080	0.062	0.035	0.063	0.055	0.080	0.287	0.120	0.006
60	0.080	0.050	0.059	0.068	0.067	0.093	0.102	0.208	0.007
72	0.138	0.073	1.722	0.074	0.096	0.139	0.105	0.178	0.005
52	0.019	0.014	0.020	0.036	0.058	0.063	0.117	0.069	0.006
68	0.050	0.100	0.074	0.035	0.068	0.048	0.012	0.036	0.005
77	0.097	0.097	0.087	0.062	0.125	0.042	0.139	0.091	0.007
13	0.080	0.077	0.058	0.121	0.167	0.129	0.167	0.162	0.006

FITC-BSA

LEPTIN



High Limit = 0.500
Low Limit = 0.050

ITC/leptin mono

ual End. AUTOMIX: OFF
50-650
0.70°C
CALIBRATION: ON
SET TEMP: OFF

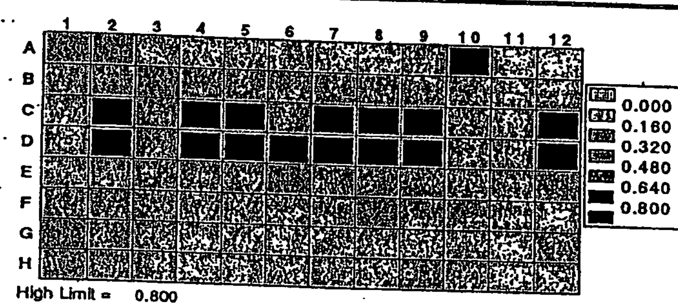
DATA FILE: FITC/leptin mono
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 20.70°C
CALIBRATION: ON
SET TEMP: OFF

Optical Density

3	4	5	6	7	8	9	10	11	12
1	0.051	0.042	0.087	0.054	0.060	0.058	1.516	0.078	0.100
2	0.046	0.043	0.059	0.053	0.050	0.045	0.040	0.131	0.082
6	1.413	1.602	0.044	1.453	1.362	1.552	0.056	0.080	1.629
7	1.547	1.639	1.405	1.494	1.341	1.497	0.087	0.051	1.626
4	0.053	0.024	0.164	0.020	0.021	0.065	0.032	0.017	0.060
4	0.040	0.071	0.084	0.042	0.165	0.062	0.061	0.034	0.076
8	0.079	0.068	0.097	0.076	0.061	0.061	0.076	0.060	0.107
6	0.108	0.100	0.071	0.144	0.072	0.057	0.075	0.063	0.077

FITC CG
DH

LEPTIN CG
DH



High Limit = 0.800
Low Limit = 0.000

E: Thy/BSA poly
N:
L:
N:
E: Dual End. AUTOMIX: OFF
H: 450-650
P: 20.50°C
CALIBRATION: ON
SET TEMP: OFF

DATA FILE: Thy/BSA poly
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 20.50°C
CALIBRATION: ON
SET TEMP: OFF

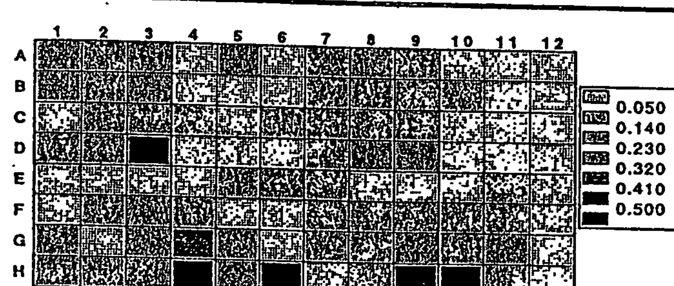
12CC 13CC 14CC 10DH 11DH 12DH 13DH 14DH

Optical Density

3	4	5	6	7	8	9	10	11	12
0.071	0.018	0.305	0.033	0.099	0.069	0.065	0.027	0.028	0.007
0.172	0.024	0.030	0.032	0.085	0.086	0.087	0.053	0.048	0.007
0.075	0.062	0.016	0.067	0.104	0.102	0.070	0.024	0.034	0.006
1.190	0.043	0.017	0.032	0.057	0.157	0.167	0.034	0.020	0.005
0.024	0.049	0.053	0.051	0.117	0.026	0.035	0.037	0.055	0.004
0.075	0.074	0.047	0.034	0.058	0.087	0.067	0.099	0.127	0.007
0.054	0.354	0.071	0.043	0.051	0.090	0.310	0.113	0.182	0.007
0.090	1.367	0.161	0.420	0.035	0.135	1.356	1.027	0.138	0.007

THY

BSA



High Limit = 0.500
Low Limit = 0.050

Thy/BSA mono 3

Dual End. AUTOMIX: OFF
450-650
20.80°C
CALIBRATION: ON
SET TEMP: OFF

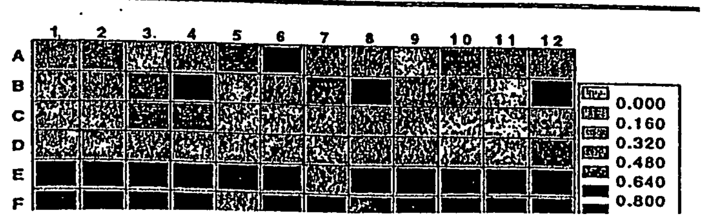
DATA FILE: Thy/BSA mono 3
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End. AUTOMIX: OFF
WAVELENGTH: 450-650
MEAN TEMP: 20.80°C
CALIBRATION: ON
SET TEMP: OFF

Optical Density

3	4	5	6	7	8	9	10	11	12
0.130	0.198	0.512	0.841	0.326	0.371	0.142	0.623	0.367	0.202
0.510	0.699	0.057	0.474	0.521	0.676	0.454	0.400	0.058	0.716
0.556	0.575	0.044	0.067	0.044	0.309	0.062	0.071	0.094	0.093
0.066	0.100	0.045	0.076	0.064	0.098	0.054	0.055	0.074	0.630
1.115	1.210	1.150	1.185	0.087	1.207	0.708	1.182	1.309	1.598

THY

BSA



Atty. Docket No.: 8039/1070 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Tomlinson, et al.	Examiner:	Ponnaluri, P.
Serial No.:	09/511,939		
Filed:	February 24, 2000	Group Art Unit:	1639
Entitled:	Method to Screen Phage Display Libraries with Different Ligands		
		Conf. No.:	5170

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF GREG P. WINTER UNDER 37 C.F.R. §1.131

I declare:

1. I, Greg P. Winter, am an inventor of the invention claimed in the above-noted U.S. Patent application.

2. I have read and understood the Office Action mailed July 11, 2003 and have read and understood the cited reference, U.S. Patent No. 6,057,098 (the "'098 patent;" issued to Buechler et al. on May 2, 2000 from an application filed April 4, 1997). I understand that the Examiner has cited the '098 patent as a novelty reference over claims 33-41 and 44-52.

The '098 patent is cited as teaching methods of producing a multivalent polypeptide display library comprising a library of phage representing tagged fusion proteins. The Examiner states that the tag can be any polypeptide with a known receptor showing high binding specificity for the tag (referring to column 7, lines 10-11). The Examiner further asserts that Buechler et al. teaches contacting the library with a receptor (which the Examiner characterizes as analogous to a generic ligand) and separating bound members of the library from unbound members to produce a sublibrary of polypeptides. The Examiner states that the selected sublibrary is then screened by contacting the library with a target and separating the library members bound to the target via their displayed polypeptides.

3. Prior to the April 4, 1997 filing date of the '098 patent, co-inventor Ian Tomlinson and I had conceived of the invention as claimed in claims 33-41 and 44-52. The invention was reduced to practice with diligence shortly thereafter. The attached Exhibit 1 consists of copies of Ian Tomlinson's notebook entries detailing the experiments, performed under our joint direction, that gave rise to the claimed invention. The dates on this exhibit have been redacted. A detailed description of these notebook entries and their relevance to the claimed invention is found in the accompanying Rule 131 Declaration of co-inventor Ian Tomlinson.

4. Co-inventor Ian Tomlinson and I had discussions on several occasions prior to April 4, 1997 regarding ways to improve polypeptide library technology. Specifically, we discussed the problems posed by the presence of a large background of library members that, due to the means used to introduce diversity (typically degenerate oligonucleotides and PCR), are not capable of proper folding and are therefore non-functional for binding to any target molecule. We recognized that a selection of library members for those capable of folding into a functional conformation would increase the likelihood of identifying library members that bind a desired target ligand.

Over the course of these ongoing discussions, the idea was developed that a functional conformation could be selected by selecting members of a library that bind a ligand generic to all functional members of the library. If proper folding is required for binding to the generic ligand, selection for such generic ligand binding would increase the proportion of molecules potentially in a conformation capable of binding a desired target. Selection performed on the resulting enriched library can identify functional members that also bind a desired target ligand. Thus, we had conceived of an approach for selecting a functional polypeptide library member from a repertoire comprising functional and non-functional members by selecting the repertoire with a generic ligand that only binds functional members, and then selecting the resulting pool of functional members for binding to a target ligand. One specific example of generic ligand binding we initially discussed was the binding of antibody molecules by superantigens, such as protein A and protein L. These proteins require that the antibody molecule be properly folded before they can bind. Therefore, such superantigen binding can be used to select library members that are properly folded.

We also recognized that the selection for folded, functional library members would have a particular impact on antibody libraries, because antibodies that have both Heavy and Light chains require the proper folding of both chains before they can bind a desired target ligand. Selection of a first selected pool of Heavy and/or Light chain polypeptides enriched for functional polypeptides, would be expected to increase the proportion of molecules potentially able to also bind a desired target ligand. One could then contact the first selected pool of polypeptides with a target ligand to select a population of polypeptides which bind to the target ligand. Thus, in our discussions prior to April 4, 1997, we conceived an approach to an improved antibody library in which a sub-library of antibody Heavy chains is selected for members that properly fold, a sub-library of Light chains is selected for members that properly fold, and the two selected sub-libraries are combined to form a library of antibodies that have been pre-selected for folded members that is then selected for binding to target antigen. The pre-selection for proper folding is performed by binding the members of each sub-library to a generic ligand that only binds properly folded sub-library molecules. For example, the Heavy chain sub-library can be pre-selected with Protein A, and the Light chain sub-library can be pre-selected with Protein L.

To bring our conception to fruition, Ian Tomlinson and I discussed, before April 4, 1997, the approach in which two scFv vector constructs are made, one encoding a "dummy" Heavy chain to be used to generate a sub-library of diverse Light chains, and one encoding a "dummy" Light chain to be used to generate a sub library of Heavy chains. Following selection for proper folding of the diverse domains by generic ligand binding of both sub-libraries, the "dummy" domain of one sub-library was to be replaced with the corresponding folding-selected domains from the other sub-library to generate a library of diverse, properly folded scFvs that could be selected for target binding.

Beginning before April 4, 1997, the experiments required to demonstrate this approach were undertaken under the direction of Ian Tomlinson. He and I conferred during the progress of the experiments until their successful completion after April 4, 1997, described in the notebook pages accompanying Dr. Tomlinson's Rule 131 declaration being filed concurrently with this declaration. I have reviewed the notebook pages and fully concur that our discussions and Dr.

Tomlinson's experimental work are in accord with both the notebook entries and Dr. Tomlinson's statements in his declaration.

5. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

22/9/03

Date

G. P. Winter

Greg P. Winter